47. **(new)** A *Pichia* expression vector comprising:

a first and a second expression cassette, said first cassette comprising a first promoter operably linked to a nucleic acid encoding an immunoglobulin light chain operably linked to a first signal peptide, and said second cassette comprising a second promoter operably linked to a nucleic acid encoding an immunoglobulin heavy chain operably linked to a second signal peptide,

wherein introduction of said vector into a *Pichia* host cell provides for production of a recombinant immunoglobulin protein that specifically binds an antigen and is secreted by the host cell.

48. **(new)** A recombinant *Pichia* cell containing the expression vector of claim 47.

49. **(new)** A method for production of an antibody comprising the steps of:

culturing the recombinant *Pichia* cell of claim 48 so as to provide for antibody expression; and

harvesting the antibody from culture supernatant.

II. REMARKS

Formal Matters

Claims 36-49 are pending after entry of the amendments set forth herein.

Claims 22 and 25-32 were examined. Claims 22 and 25-32 were rejected.

Claims 22 and 25-32 are canceled without prejudice to renewal, without intent to acquiesce to any rejection, and without intent to surrender any subject matter encompassed by the canceled claims. Applicants expressly reserve the right to pursue any canceled subject matter in one or more continuation and/or divisional applications.

Support for the new claims 36-49 may be found in, for example, the claims as originally filed.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

Current status of claims

Our review of the prosecution history of this case indicates claims 22 and 25-32 are pending, and that two rejections are maintained:

Claims 22 and 27-32 are rejected under 35 U.S.C. §103(a) as obvious over Horwitz in view of Cregg, the Invitrogen 1997 Catalog, and Sambrook.

Claims 25 and 26 are rejected under 35 U.S.C. §103(a) as obvious over Horwitz in view of Cregg, the Invitrogen 1997 Catalog, Sambrook and Vanderlaan.

Summary of Pending Claims

Claims 36-49 are pending after entry of the amendment set forth above. For the Examiner's convenience, we provide below a summary of the subject matter of the presently pending claims. This summary is not intended to be limiting as to the scope of the claimed subject matter, but rather is merely to provide a brief description of the distinctions among the various claim sets.

Methods of producing antibody

New claims 36-46 and 49 are directed to a method of producing antibody in *Pichia* using a *Pichia* expression vector containing dual expression cassettes for expression of a heavy and light chain immunoglobulin. Claim 41 recites an anti-dioxin antibody.

Expression vectors

Claim 47 is directed to a *Pichia* expression vector comprising dual expression cassettes for expression of a heavy and light chain immunoglobulin.

Recombinant Pichia

Claim 48 is directed to recombinant *Pichia* comprising the expression vector of claim 47.

Outstanding rejections

The prosecution history of this case indicates that all pending claims have been rejected as obvious under 35 U.S.C. §103(a) over Horwitz in view of a number of secondary references. These rejections are addressed below.

Rejections under 35 U.S.C. §103

Claims 22 and 27-32 have been rejected under 35 U.S.C. §103(a) as obvious over Horwitz *et al* (PNAS 85:8678-8682) in view of Cregg *et al* (Developments in Industrial Microbiology 29:33-41, 1998), The Invitrogen 1997 Catalog, and Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, Second Edition, 1989), for the asserted reason that Horwitz discloses a vector system and method for production of functional antibodies in *S. cerevisiae*, which, when combined with Cregg's *Pichia* alcohol oxidase promoter, Invitrogen's *Pichia* vector system, and the general cloning methodologies of Sambrook, renders the claims obvious to one of skill in the art.

Claims 22 and 27-32 are canceled. For the purposes of this response, the above rejection of claims 22 and 27-32 under 35 U.S.C. §103(a) is addressed as it may be applied to claims 36-40 and 42-46.

The Office has taken the position, as determined above, that claims 22-24 and 27-32 are obvious under 35 U.S.C. §103(a) over Horwitz in view of a number of secondary references. Applicants respectfully traverse the rejection as it applied or as it may be applied to pending claims 36-40 and 42-49.

The M.P.E.P. teaches at §1242 that:

> To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, whether in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

Thus, in order for a proper *prima facie* case to be established, a combination of references must teach or suggest all of the claim limitation.

As will be demonstrated below, the references cited by the Office Action do not teach or suggest each and every element of the claims as pending. Accordingly, a proper *prima facie* case of obviousness has not been made.

The instant application discloses and claims vectors and methods for using the vectors for producing recombinant *Pichia* cells and antibodies. An element of each of the claims is a vector containing first and second expression cassettes (i.e. "dual expression cassettes") for the expression of light chain and heavy chain antibody subunits, respectively, in *Pichia*. Claims 36-49 recite a *Pichia* cell

a vector comprising a first and a second expression cassette. As such, all claims under examination recite a vector containing dual expression cassettes for expression of both heavy and light chain subunits of an antibody.

Horwitz discloses yeast immunoglobulin expression vectors for use in expressing recombinant antibodies in yeast. Horwitz discloses use of two vectors, one vector to express an immunoglobulin heavy chain and the other vector to express an immunoglobulin light chain (See Figure 2). Both vectors are introduced into yeast. That a single vector which can be used to express heavy and light chain subunits of an antibody is neither taught nor suggested by Horwitz.

The Invitrogen Catalog discloses several single cassette vectors designed for simple cloning and expression of a single gene of interest. These vectors include pPICZ (containing an OAX promoter and a polylinker), pPICZα (containing an AOX promoter, a *Saccharomyces* α-factor secretion signal and a polylinker), and pPIC9K, pPIC3.5K, pAO815 that contain a selection of polylinkers and selectable markers. None of the vectors disclosed in the cited pages of the Invitrogen Catalog have a dual expression cassette suitable for the expression of heavy and light chain subunits of an antibody. As such, the vectors of the Invitrogen catalog are only suitable for the expression of single polypeptides, such as those listed on page 14 of the catalog.

Horwitz and the Invitrogen Catalog are deficient because they do not teach a dual expression cassette vector for production of antibody heavy and light chains. Cregg and Sambrook fail to overcome this deficiency because they do not teach or suggest *Pichia* vectors having, within the same vector, dual expression cassettes.

Furthermore, one of skill in the art would not have any reasonable expectation of success in expressing two subunits of an antibody using one vector because of the problems associated with intra-molecular recombination (*e.g.* occurring when two parts of a vector are related), transcriptional interference (*e.g.* occurring when transcription of one cassette reads through an interferes with the transcription of the second cassette), and translational interference (*e.g.* occurring when transcriptional read-through of one cassette produces an antisense molecule that interferes with the translation of the RNA from the second cassette). Such problems are commonly associated with such dual cassette vectors, especially when the expression cassettes contain polynucleotides with similar or identical sequences (for example the same promoters, signal sequence-encoding polynucleotides or terminators).

Combining Horwitz with the disclosures of Cregg, the Invitrogen Catalog and Sambrook does not provide any suggestion of a *Pichia* expression vector having such dual expression cassettes, and one of skill in the art would not have a reasonable expectation of success in making and using such a dual

cassette vector. Because the instant claims recite dual expression cassette vectors, the instant claims cannot be made obvious by the cited publications.

As such, claims 36-40 and 42-49 are not made obvious under 35 U.S.C. §103(a) over Horwitz in view of Cregg, the Invitrogen 1997 Catalog, and Sambrook. Accordingly, these rejections may be withdrawn.

Claims 25 and 26 have been rejected under 35 U.S.C. §103 as obvious over Horwitz *et al* (PNAS 85:8678-8682) in view of the secondary references described above, in further view of Vanderlaan *et al* (US Patent 5,429,925) for the asserted reason that Horwitz discloses a vector system, which, when combined with Cregg's promoter, Invitrogen's *Pichia* vector system, the methodologies of Sambrook, and the dioxin antibody of Vanderlaan, renders the claims obvious to one of skill in the art.

The above rejection of claims 25 and 26 under 35 U.S.C. §103(a) is addressed as it may be applied to claim 41.

The Office Action stated, as determined above, that claims 41 are obvious under 35 U.S.C. §103(a) over Horwitz in view of a number of secondary references, including the Vanderlaan reference, which teaches an anti-dioxin antibody.

Applicants assert above that Horwitz, the Invitrogen Catalog, Cregg and Sambrook are fundamentally deficient in not teaching dual cassette expression vectors for production of antibody heavy and light chains. Vanderlaan, which is cited solely because it teaches a monoclonal anti-dioxin antibody, also does not make up for this deficiency.

Combining Horwitz with the disclosures of Cregg, the Invitrogen Catalog, Sambrook and Vanderlaan does not provide any suggestion to provide a *Pichia* expression vector having such dual expression cassettes for production of antibody heavy and light chains, and one of skill in the art would not have a reasonable expectation of success in making and using such a dual cassette vector. Because the instant claims recite dual expression cassette vectors, the instant claims cannot be made obvious by the cited publications.

As such, claim 41 is not made obvious under 35 U.S.C. §103(a) over Horwitz in view of Cregg, the Invitrogen 1997 Catalog, Sambrook and Vanderlaan. Accordingly, this rejection may be withdrawn.

Vector, host cells and methods of using the same

Before they were canceled in the amendment filed on August 17, 2001, claims 33-35 were rejected under 35 U.S.C. §103(a) as obvious over Horwitz *et al* in view of The Invitrogen 1997 Catalog for the asserted reason that Horwitz discloses a vector system and method for production of functional antibodies in *S. cerevisiae*, which, when combined with Invitrogen's *Pichia* vector system renders the claims obvious to one of skill in the art.

Claims 33-35 are canceled. For the purposes of this response, the above rejection of claims 33-35 under 35 U.S.C. §103(a) is addressed as it may be applied to claims 47-49.

Claim 47 recites a dual cassette vector for expressing immunoglobulin heavy and light chains in *Pichia*. Claim 48 recites a recombinant *Pichia* cell containing the vector of claim 47, and claim 49 recites a method of culturing the recombinant *Pichia* cell of claim 48 to make active antibodies.

As asserted above, Horwitz and the Invitrogen Catalog, either taken singly or in combination, do not teach or suggest a dual expression cassette vector suitable for expression of immunoglobulin heavy and light chains, and one of skill in the art would not have a reasonable expectation of success in making and using such a dual cassette vector.

As such, claims 47 are not made obvious under 35 U.S.C. §103(a) over Horwitz in view the Invitrogen Catalog. Should claim 47, reciting a dual expression cassette vector become allowable, Applicants respectfully submit that claims 47 and 48, reciting methods of using the vector of claim 47, should likewise become allowable. Accordingly, should claim 47 become allowable, the rejections of claims 48 and 49 may be withdrawn.

III. CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number UCAL-269.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: May 10, 2002

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Claims 22 and 25-32 are canceled.

Claims 36-49 are newly added.